

Geiger-Müller tube which was standardized with material of known specific activity. Corrections for geometry and self-absorption were made and the results are expressed as disintegrations per second.

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OAK RIDGE, TENNESSEE

[CONTRIBUTION FROM THE WHITMORE LABORATORIES OF THE COLLEGE OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE UNIVERSITY]

## Synthesis and Properties of Deuterocarbons. Benzene- $d_6$ and Cyclohexane- $d_{12}$ <sup>1a</sup>

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Samples of benzene- $d_6$  and cyclohexane- $d_{12}$  of up to 99.3 and 98.8% deuteration, respectively, have been prepared by stepwise equilibration of benzene with sulfuric acid- $d_2$ , followed by reduction with deuterium over nickel catalyst for conversion to the cyclohexane- $d_{12}$ . Density and refractive index of these materials at 20, 40 and 60° have been measured, and molar volumes and refractions calculated. The physical properties are compared with those of the protium isomers. A relation between the density-temperature coefficients and the molecular weights of the isotope pairs is noted, and some possible implications suggested.

Present theories of the liquid state are extremely complex and unfortunately always involve several quantities which cannot be determined or estimated accurately.<sup>2-4</sup> It follows, of course, that prediction of liquid properties (*e.g.*, viscosity) suffer from the same disadvantages, and the calculation of physical properties from first principles for a relatively simple normal paraffin molecule is beyond the ability of present techniques. Quantitative knowledge of the contribution of the various parameters of liquid properties could be of considerable value in bridging this gap.

Comparison of deuterocarbons and their hydrocarbon analogs should be useful in determining the magnitude of the mass parameter for liquid properties. Apparently replacement of hydrogen by deuterium in an organic molecule little affects the outer molecular force fields or the molecular volume. To begin this study, benzene- $d_6$  and cyclohexane- $d_{12}$  have been prepared and their physical properties determined.

Although the literature on deuterated compounds is voluminous,<sup>5</sup> the only important work relating to the effect of deuterium on liquid properties was reported by Ingold, Wilson and co-workers.<sup>6</sup> These authors studied benzene- $d_6$  in which 99.1% of the hydrogen was replaced by deuterium.

### Experimental

**Reagents.**—Deuterium oxide and deuterium were obtained from the Stuart Oxygen Company and specified to have a minimum deuterium percentage of 99.5.

(1) (a) Presented before the XIIth International Congress on Pure and Applied Chemistry, New York, N. Y., September, 1951. Part of investigation by American Petroleum Institute Research Project 42. (b) Department of Chemistry, Lafayette College, Easton, Pa.

(2) S. Glasstone, K. J. Laidler and H. Eyring, "Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941.

(3) M. Born and H. S. Green, "General Kinetic Theory of Liquids," Cambridge University Press, 1949.

(4) I. I. Frenkel, "Kinetic Theory of Liquids," Clarendon Press, Oxford, 1946.

(5) A. H. Kimball, "Bibliography of Research on Heavy Hydrogen Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1949.

(6) C. K. Ingold, C. G. Raisin, C. L. Wilson, C. R. Bailey and B. Topley, *J. Chem. Soc.*, 915 (1936); see also L. H. P. Weldon and C. L. Wilson, *ibid.*, 235 (1946).

The benzene was Phillips Petroleum Company Research Grade 99.93 ( $\pm 0.03$ ).

The sulfur trioxide was supplied by the General Chemical Company and was stated to be better than 99%.

The nickel catalyst was a kieselguhr-supported, pelleted material containing 60% nickel, supplied by the Universal Oil Products Company. The catalyst was reduced *in situ* before starting the exchange experiments, and again whenever the exchange rate decreased significantly. Reduction was with hydrogen at 200–250° for eight hours.

**Reaction of Benzene and Water.**—The all-glass apparatus used in studying this reaction consisted of a boiler, a heated catalyst chamber and a condensing system. In operation, the boiler continuously supplied an azeotropic mixture of benzene and water to the catalyst. After passage through the catalyst chamber the vapors were condensed and returned to the boiler. At the completion of an equilibration the water and benzene were separated. The recovered water was purified by distillation, and the benzene by distillation from powdered calcium hydride.<sup>7</sup> Isotopic compositions were calculated from the densities of the products, and were later determined from mass spectra.

**Preparation of Benzene- $d_6$ .**—The procedure is similar to that of Ingold.<sup>6</sup> The heavy sulfuric acid was prepared by distilling sulfur trioxide at 50–100 mm. into a receiver containing deuterium oxide. Anhydride vapor readily dissolved in the magnetically-stirred heavy water.

Benzene and 51–52 mole per cent. sulfuric acid- $d_2$  were charged to a 500-cc. Pyrex bottle having a carefully ground glass closure. The mixture was shaken vigorously for ten days at room temperature in a dry nitrogen atmosphere. The layers separated readily on standing and the sulfuric acid was pipetted from the bottle. When desired, an aliquot of the benzene was removed and distilled from powdered calcium hydride in an all glass system. This procedure removed water, sulfuric acid and benzenesulfonic acids in one step. The equilibration was repeated with fresh portions of sulfuric acid- $d_2$  until a benzene having the desired deuterium content was obtained. The equilibrium distribution of deuterium between acid and benzene at room temperature was found to be nearly statistical. The number of equilibrations required depends on the mole ratio of acid to benzene used and the desired deuterium concentration of the benzene.

**Preparation of Cyclohexane and Cyclohexane- $d_{12}$ .**<sup>8</sup>—The reduction of the appropriate benzene with hydrogen or deuterium was carried out over kieselguhr-supported nickel at 400–700 p.s.i. and 100–125° in an Aminco apparatus using a stainless steel liner. The cyclohexane was made by hydrogenation of Phillips Research Grade benzene. The

(7) It was established that no exchange of hydrogen occurs between calcium hydride and benzene under such conditions.

(8) The preparation of perdeuterocyclohexane is reported by A. Langseth and B. Bak, *J. Chem. Phys.*, **8**, 1103 (1940), boiling point 77.8–78.0°.

TABLE I

	D, %	Density (g./cc.)			$n_D^t$			At 20°	
		20°	$10^5$	60°	20°	30°	40°	Molar <sup>a</sup> refraction	Molar <sup>a</sup> volume
Benzene	0.0	0.8790	0.8574	0.8359	1.50110	1.49478	1.48844	26.18	88.86
Benzene- $d_6$	99.3	.9488	.9257	.9022	1.49910	1.49278	1.48645		
Benzene- $d_6^b$	100.0	.9494	.9263	.9028	1.49911	1.49279	1.48646	26.03	88.63
Cyclohexane	0.0	.7787	.7596	.7406	1.42630	1.42088	1.41525	27.71	108.07
Cyclohexane- $d_{12}$	98.8	.8913	.8691	.8470	1.42185	1.41632	1.41078		
Cyclohexane- $d_{12}$	100.0	.8927	.8704	.8483	1.42181	1.41627	1.41073	27.38	107.80

<sup>a</sup> Atomic weight of deuterium, 2.0147. <sup>b</sup> These values are in excellent agreement with data reported in reference 6.

cyclohexane- $d_{12}$  was obtained from deuterogenation of the benzene- $d_6$ .

### Discussion

The preparation of benzene- $d_6$  by equilibration of deuterium oxide and benzene over a kieselguhr-supported nickel at 200° was reported by Taylor, *et al.*<sup>9</sup> Their method is attractive for its speed and apparent simplicity, and was reinvestigated in the present work. Unfortunately we discovered that in addition to the hydrogen-deuterium exchange there are at least two other competing reactions, making the method unsuitable for preparing high purity benzene- $d_6$ . A gas was evolved which was<sup>10</sup> composed of 60% hydrogen and 24% carbon dioxide. The gas was produced at temperatures as low as 150°. This reaction of benzene with water to produce carbon dioxide and hydrogen resembles the low temperature water gas reaction.

In addition, a trace of white solid, apparently biphenyl, was separated from benzene which had been cycled through the catalytic chamber at 150–175° many times. The cracking of aromatic and aliphatic hydrocarbons on metal or metal oxide catalysts at higher temperatures (300 to 1200°) is well-known.<sup>11,12</sup>

The equilibration of benzene and 51–52 mole per cent. sulfuric acid- $d_2$  has been shown<sup>6</sup> to produce deuterium substitution with no carbon skeletal rearrangements, and the present work fully confirms the report. The distribution constant<sup>13</sup> for the exchange at 20 ( $\pm 2^\circ$ ) was found to be 1.12 ( $\pm 0.05$ ). The benzene- $d_6$  sample on which the physical properties were determined contained 99.3 ( $\pm 0.1\%$ ) deuterium, as determined by mass spectra<sup>14</sup> and density. Mass spectrometer analyses show that density is linear with deuterium content to within one per cent. Combustion of the benzene followed by determination of the density of the deuterium oxide led to the same conclusion by Ingold, Wilson and co-workers.<sup>6</sup>

Interpreting the mass spectra, Dr. Beach<sup>14</sup> reported: "It was possible to determine not only the per cent. hydrogen replaced by deuterium but also concentrations of all the successively substituted

(9) P. I. Bowman, W. S. Benedict and H. S. Taylor, *THIS JOURNAL*, **57**, 960 (1935).

(10) We are indebted to F. P. Hochgesang, Socony-Vacuum Laboratories, Paulsboro, New Jersey, for this mass spectrometer analysis.

(11) C. Ellis, "The Chemistry of Petroleum Derivatives," Gwinn and Company, New York, N. Y., 1934.

(12) H. M. Stanley, "The Science of Petroleum," Vol. III, Oxford University Press, London, 1938.

(13) A. I. Bordskii, *Trans. Faraday Soc.*, **33**, 1180 (1937).

(14) We are indebted to J. Y. Beach, California Research Corporation, Richmond, California, for the mass spectra analyses of the samples having 30, 45, 70, 95 and 99.3% hydrogen replaced by deuterium.

isomers. This includes  $C_6H_6$ ,  $C_6H_3D_3$ ,  $C_6H_4D_2$ , etc., but not such isomers as *o*-, *m*- and *p*- $C_6H_4D_2$ . These concentrations were then compared with the isomeric concentrations which would be expected if there were complete statistical equilibrium of the deuterium atoms among all the possible isomers. Such calculations for all deuterium contents led to the over-all mole fraction of deuterium (with respect to H and D) for each sample and also showed that each sample consisted within narrow limits of a complete statistical equilibrium mixture of all the expected deuterated isomers."

In Table I are listed the physical properties determined<sup>15</sup> for the pertinent samples. The same properties were determined for benzene samples having 30, 45, 70 and 95% hydrogen replaced by deuterium, and, for these statistically-distributed mixtures, the physical properties varied linearly with deuterium content.

The relatively large change in refractive index, approximately  $200 \times 10^{-5}$  unit for benzene and  $400 \times 10^{-5}$  unit for cyclohexane, makes this convenient property useful for following the conversion of the hydrocarbon to the deuterocarbon. The decrease in molecular refractivity though small is real, and may be interpreted as a lowering of the static polarizability of the molecule.<sup>16</sup> The decrease in molar volume has been ascribed previously to the decrease in the amplitude of the zero point vibration of the deuterocarbon.<sup>6</sup>

The temperature coefficients of density  $\partial\rho/\partial T = \alpha$ , and of refractive index  $\partial n/\partial T = \beta$ , are listed in Table II. The refractive index coefficients are identical for each isotopic pair, but the density coefficients of the deuterocarbons are considerably greater than those of the hydrocarbons. At first surprising, this result became more interesting when it was noted that the ratio of the density-temperature coefficients for an isotopic pair was nearly equal to the ratio of molecular weights for the same pair.

	$\alpha_D/\alpha_H$	$M_D/M_H$
Benzene	1.08	1.08
Cyclohexane	1.16	1.14

TABLE II

	$(\partial\rho/\partial T) = \alpha$ , g./cc./°C.	$(\partial n/\partial T) = \beta$
$C_6H_6$	$-1.077 \times 10^{-3}$	0.00633
$C_6D_6$	$-1.165 \times 10^{-3}$	.00633
$C_6H_{12}$	$-0.955 \times 10^{-3}$	.00553
$C_6D_{12}$	$-1.110 \times 10^{-3}$	.00554

(15) F. C. Whitmore, L. H. Sutherland and J. N. Cosby, *THIS JOURNAL*, **64**, 1360 (1942).

(16) This explanation was also offered on page 918 of reference 6.

Further consideration suggested an involvement of the temperature coefficient of molar volume, and it was noted that the molar volumes of the isotopic pairs differ by a constant 0.25 ( $\pm 0.02$ ) cc./mole at both 20 and 40°, indicating that the molar volume decrement accompanying 100% replacement of protium by deuterium is little affected by temperature, as would be expected. Algebraic examination of the mathematical relations, too detailed for inclusion here, showed that since the density-temperature curve is nearly linear for these substances over a 20° span, the difference in density between 20 and 40° is proportional to the density-temperature coefficient for an individual compound, and if the difference in isothermal molar volumes for an isotopic pair is small compared to

the molar volumes themselves, the approximate proportionality between the density-temperature coefficient ratios and the molecular weight ratios would be expected.

These findings suggested that the temperature coefficients of molar volume for hydrocarbon series having constant values of "x" in the empirical formula  $C_nH_{2n+x}$  might be related to the molecular weights by a simple expression. This hypothesis has been tested and the results show a convenient linear relationship which will be the subject of a separate publication.

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[CONTRIBUTION FROM THE SCHOOL OF PHARMACY, UNIVERSITY OF WISCONSIN]

## Metal Chelates of Riboflavin<sup>1</sup>

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A number of metal chelates of the isoalloxazines, riboflavin and 6,7-dichloro-9-(1'-D-sorbityl)-isoalloxazine, have been prepared and isolated. Evidence for chelate formation is provided by a drop in pH during the formation, absence of metal ions in the solution, analyses of the purified products, production of characteristic colors, decreased solubility in alkaline solution and comparison with a true salt formation. The chelates show the same relative degree of insolubility as riboflavin itself.

Since the announcement of a tumor-inhibiting action by riboflavin<sup>2</sup> and later by related isoalloxazines,<sup>3</sup> little has been done to explain this activity. A number of metal salts and chelates has also been reported to be inhibitory to tumor growth,<sup>4</sup> which suggested the possibility that metal chelates of riboflavin might be involved in tumor inhibition or possibly in tumor growth. To test this idea, a number of metal chelates of riboflavin and that isoalloxazine reported<sup>3</sup> to be most active in tumor inhibition were prepared.

Previously, Albert<sup>5</sup> had demonstrated the existence of metal complexes of riboflavin in aqueous solution with a 1:1 ratio of metal to riboflavin by potentiometric titration. He also reported the formation of an insoluble, non-titratable complex which he believed to have a 2:1 ratio of metal to riboflavin, but the complex was neither isolated nor characterized.

Attempts to prepare isolatable metal chelates in quantity involved first of all the use of aqueous and alcoholic suspensions of riboflavin close to neutrality. In order to obtain relatively complete complexing, it was necessary to use a procedure similar to that of Traube,<sup>6</sup> in which mannitol was complexed with metals in aqueous alkaline solution.

(1) Abstracted from the M.S. dissertation of Winthrop E. Lange, January, 1953.

(2) G. I. Kensler, K. Sugiura, N. F. Young, C. R. Halter and C. P. Rhoads, *Science*, **93**, 308 (1941).

(3) F. W. Holly, E. W. Peel, R. Mozingo and K. Folkers, *This Journal*, **72**, 5417 (1950).

(4) K. Sugiura and S. R. Benedict, *J. Cancer Res.*, **7**, 329 (1922); C. F. Geschickter and E. E. Reid, "Approaches to Tumor Chemotherapy," A. A. A. S., Washington, D. C., 1947, p. 431.

(5) A. Albert, *Biochem. J.*, **47**, 27 (1950).

(6) W. Traube and F. Kubbier, *Ber.*, **65**, 187 (1932).

Since riboflavin is soluble in aqueous alkali only at a pH of 9 or higher, this pH was maintained by simultaneous addition of alkali with metal salt to prevent precipitation of free riboflavin. The drop in pH which occurred without addition of more alkali, incidentally, provides good evidence for chelate rather than salt formation. In addition, no test for metal ion in solution by indicators such as dithizone and ferricyanide could be obtained until slightly more than two molar equivalents of metal salt to riboflavin had been added. At this point, a paper chromatogram of the supernatant solution showed the disappearance of the yellow riboflavin band, and only an orange or brown band of metal chelate remained.

This procedure was found to give immediate precipitation of colored metal chelates with all the metals employed, although much better yields were obtained with divalent than with trivalent metals, possibly because of greater insolubility of the former. To avoid excess metal, the reaction was carried out with approximately equimolar quantities of riboflavin and metal salt. Excess riboflavin then had to be removed, and since the chelates were insoluble in water, alkali and organic solvents and decomposed in either organic or mineral acids Soxhlet extraction with acetone was found the only means possible. Washing with water was found to remove adsorbed sodium as well.

Analyses for carbon, hydrogen and metal of the chelates, as shown in Table II, confirmed a structure containing two metal atoms per riboflavin molecule for each metal employed. The presence of water of hydration, to fill the coordination capacities of the metals, was indicated by the loss of